Metagenomic Analysis

An overview of the sequencing tools

The jargon

- Raw reads
- Unassembled vs assembled
- Read based
- K-mer
- Reference

The objecive

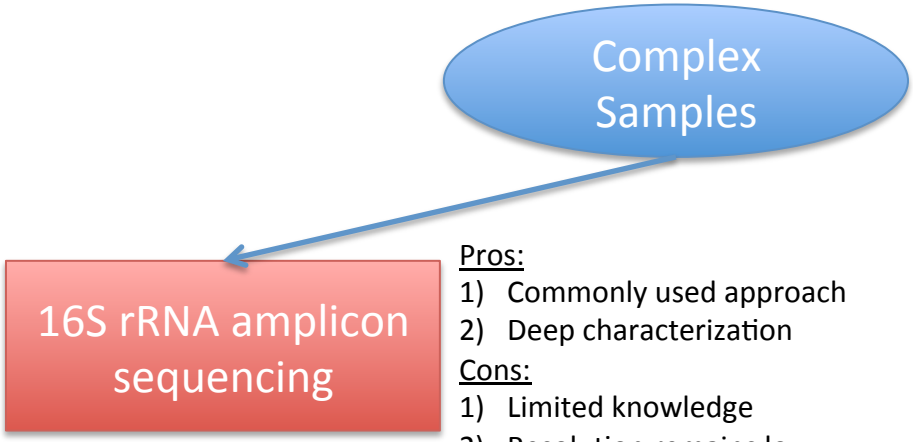
- 16S rRNA amplicon
  - OTU table (counts per “species”)
  - Classification (“species” taxonomy)
  - Metadata (sample specific information)

The objecive

- Metagenomic
  - Gene/genome count table (counts per “species”)
  - Classification (gene taxonomy/function)
  - Metadata (sample specific information)

Getting the most out of your data

ID, Abundance, Function



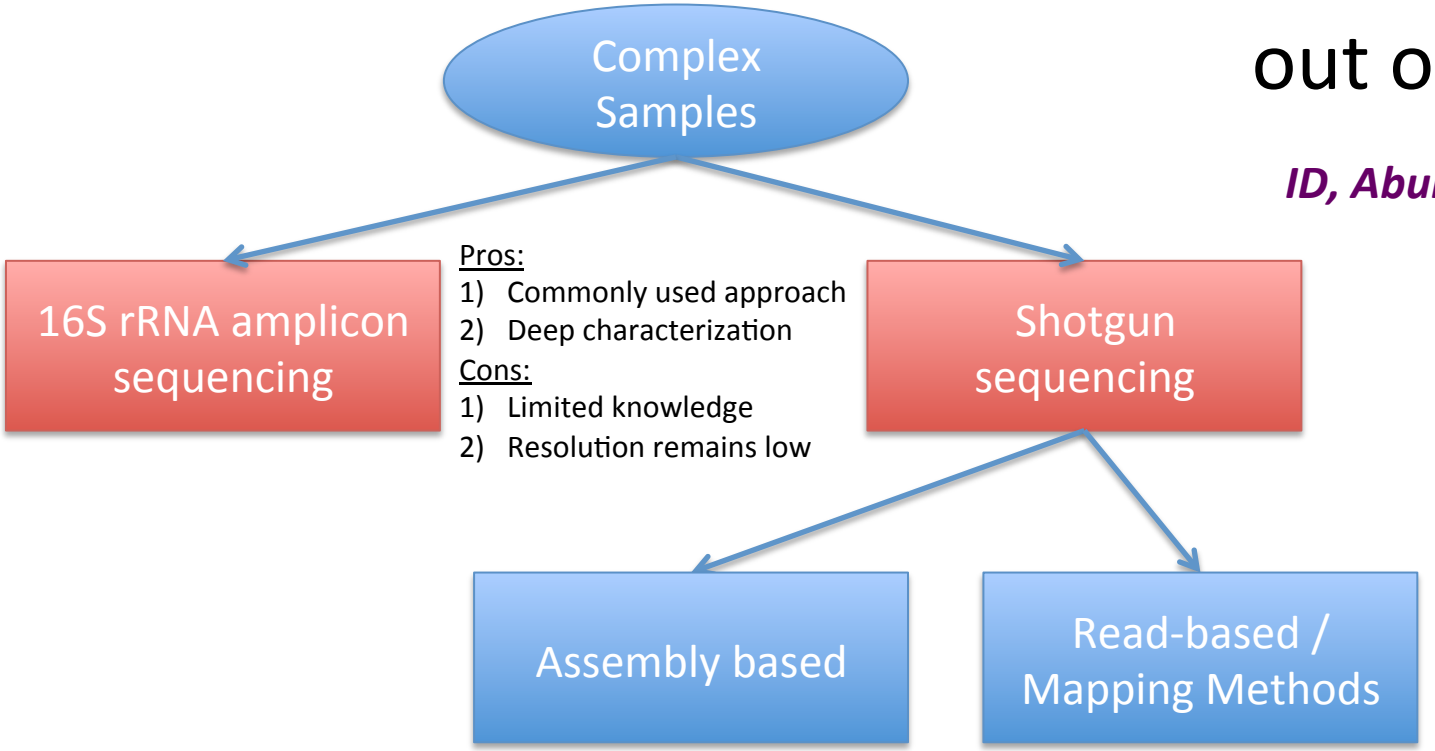
Pros:
1) Commonly used approach
2) Deep characterization

Cons:
1) Limited knowledge
2) Resolution remains low

Patrick Chain – props for these slides

Getting the most out of your data

ID, Abundance, Function



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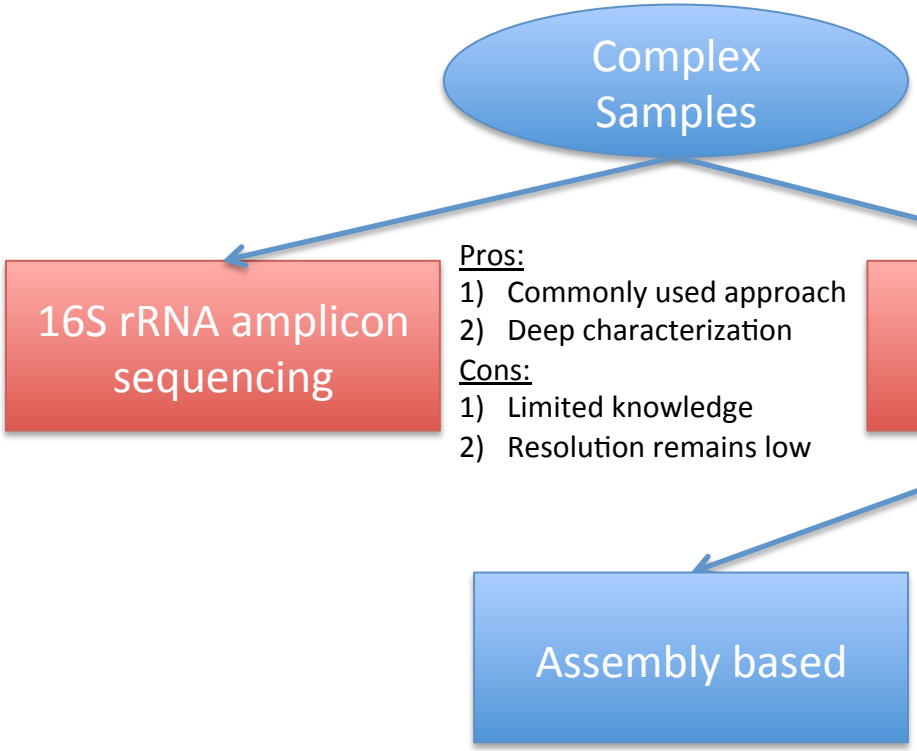

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1) Massive data
2) Identity and abundance answered simultaneously
3) Look at all data**

Cons:
1) Massive data (short + with errors)
2) Lack of specificity due to FPs from genomic redundancy
3) Difficult to detect novel genomes – must infer

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Assembly based

Pros:
1) Large contigs
2) Positional Information
3) Most direct method to identify novel orgs/genes

Cons:
1) Computational resource intensive
2) Assembling difficulties
   - Sequencing error
   - genomic redundancy - chimeras

Read-based / Mapping Methods

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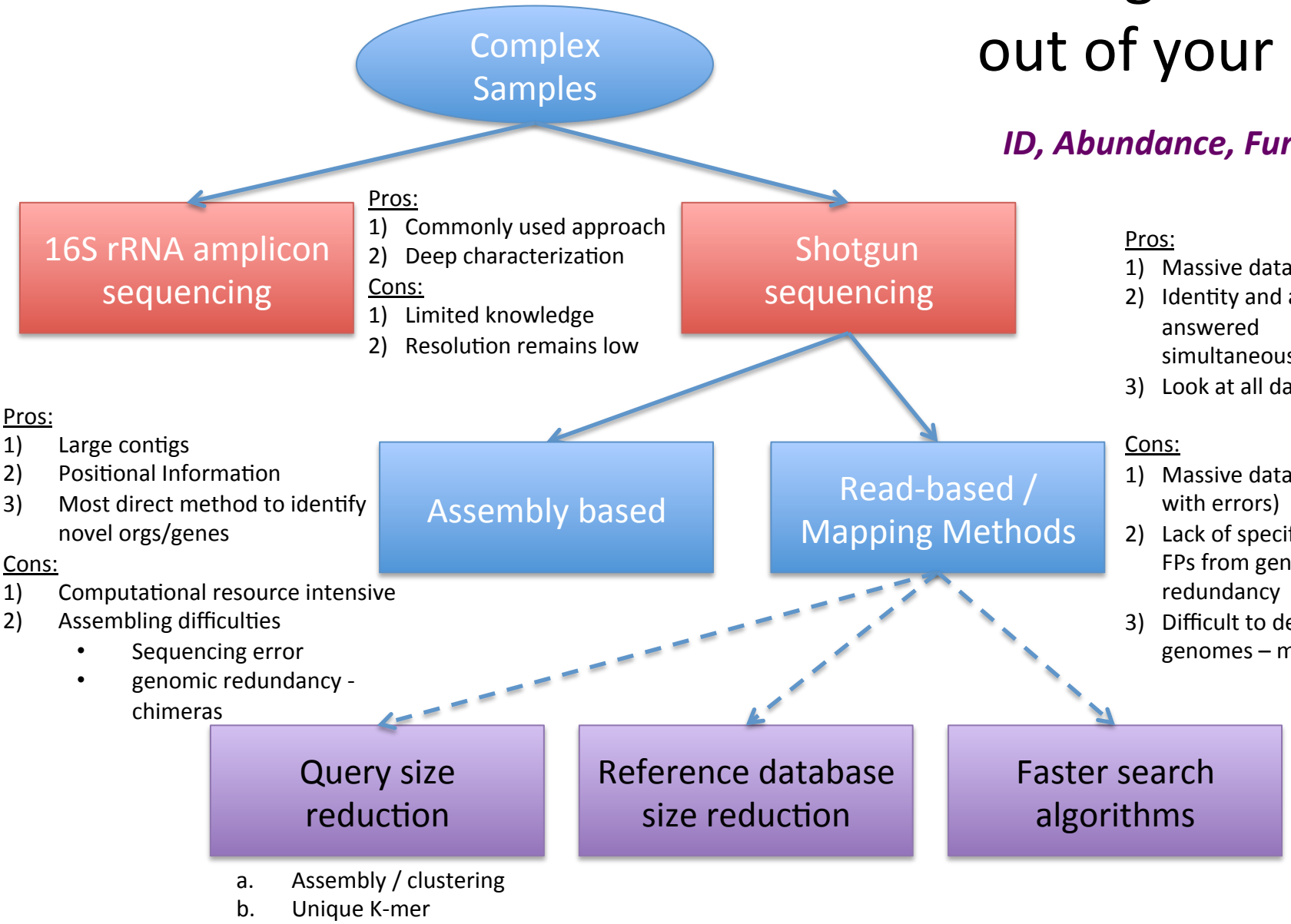

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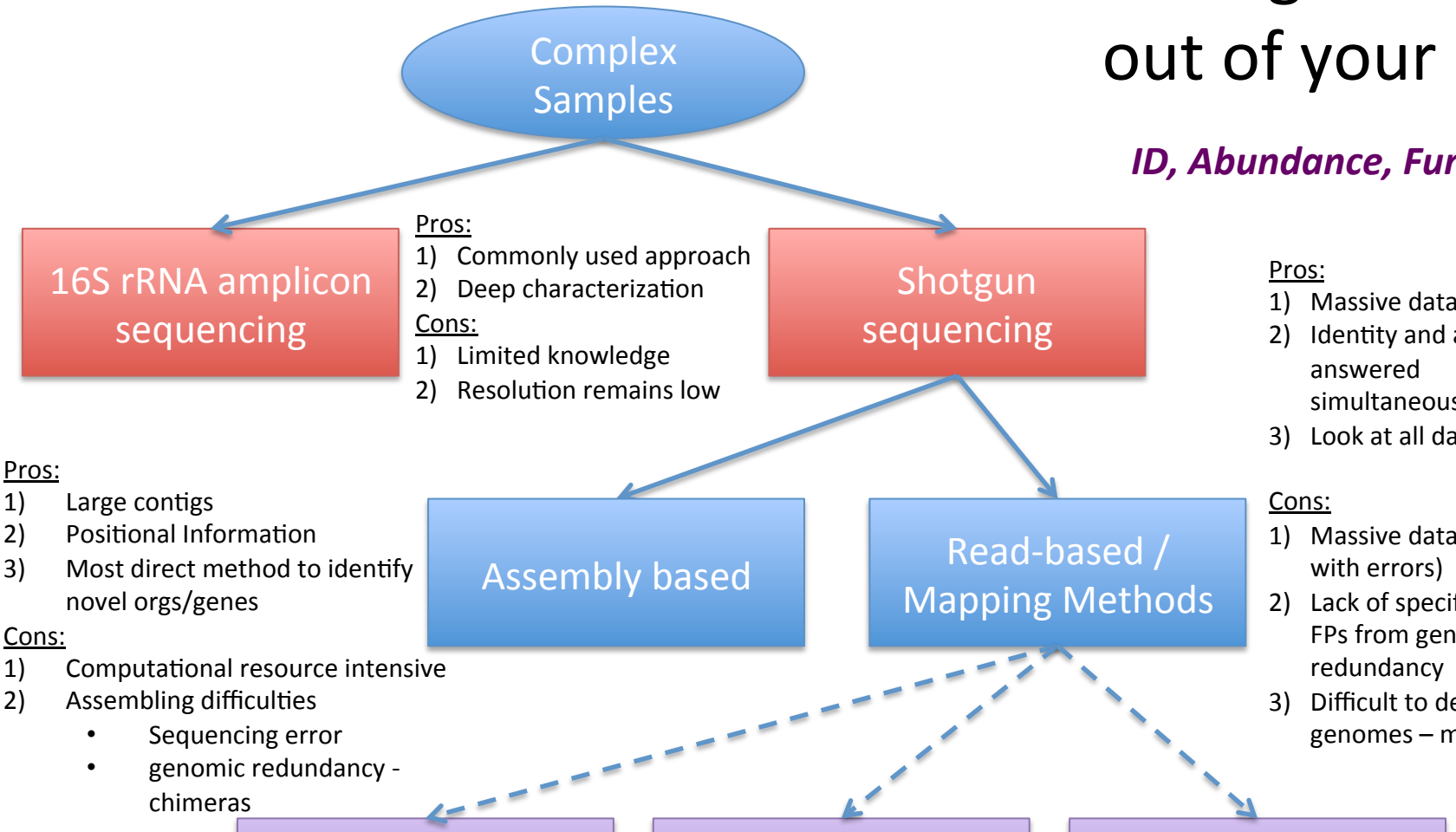

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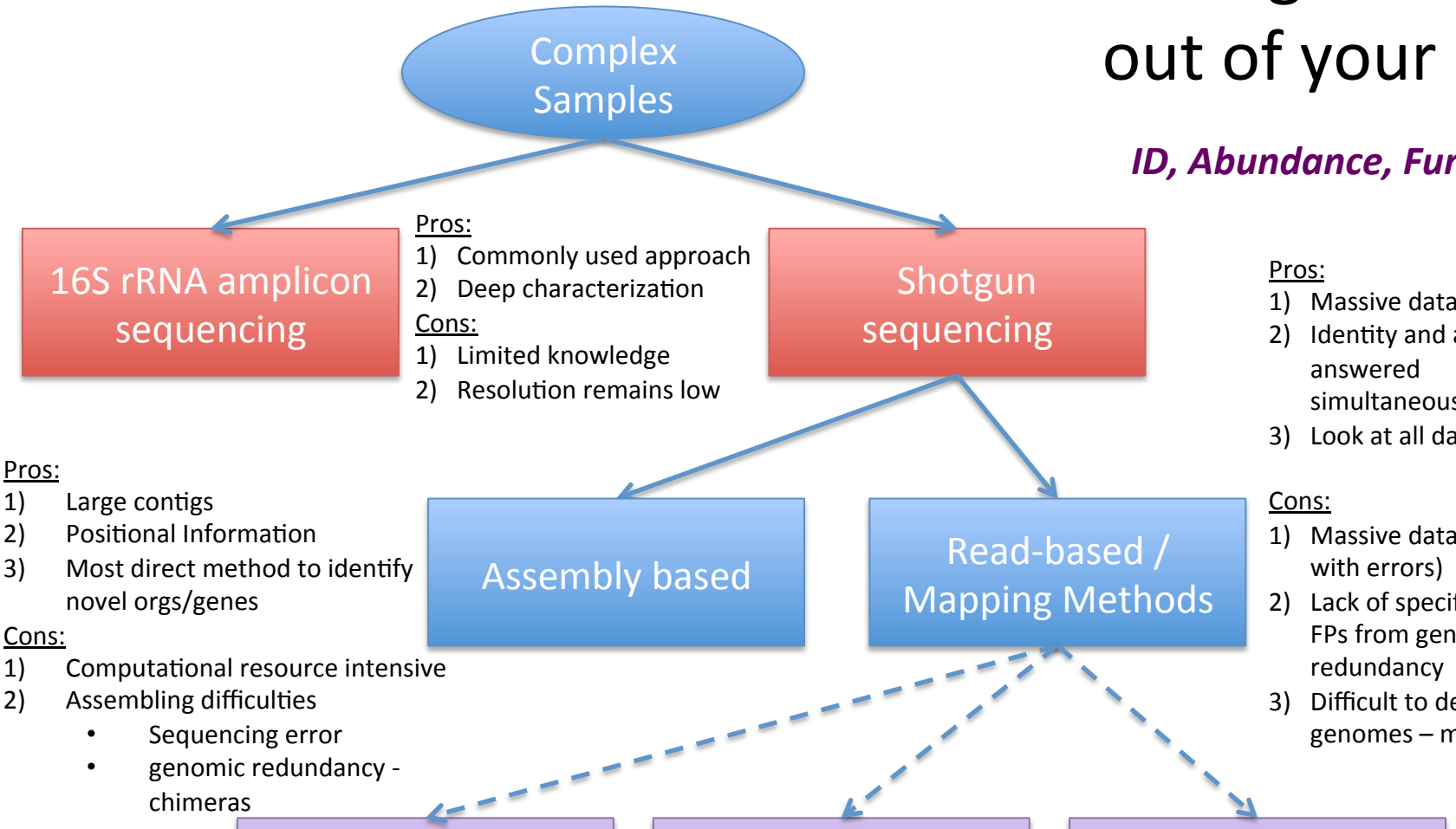

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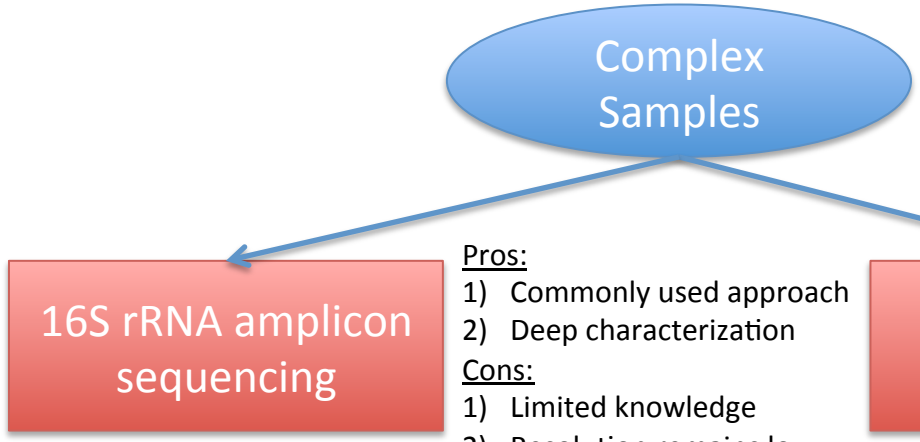

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Tutorial One: Quality trim your unassembled reads

Read-based / Mapping Methods

Query size reduction	Reference database size reduction	Faster search algorithms

a. Assembly / clustering
b. Unique K-mer
	a. Selection of marker genes
	b. Identification of signatures (Kmers)
		a. Exact match
		b. K-mer based search
		c. Improved algorithm
		  a. Clustering
		  b. Pattern matching

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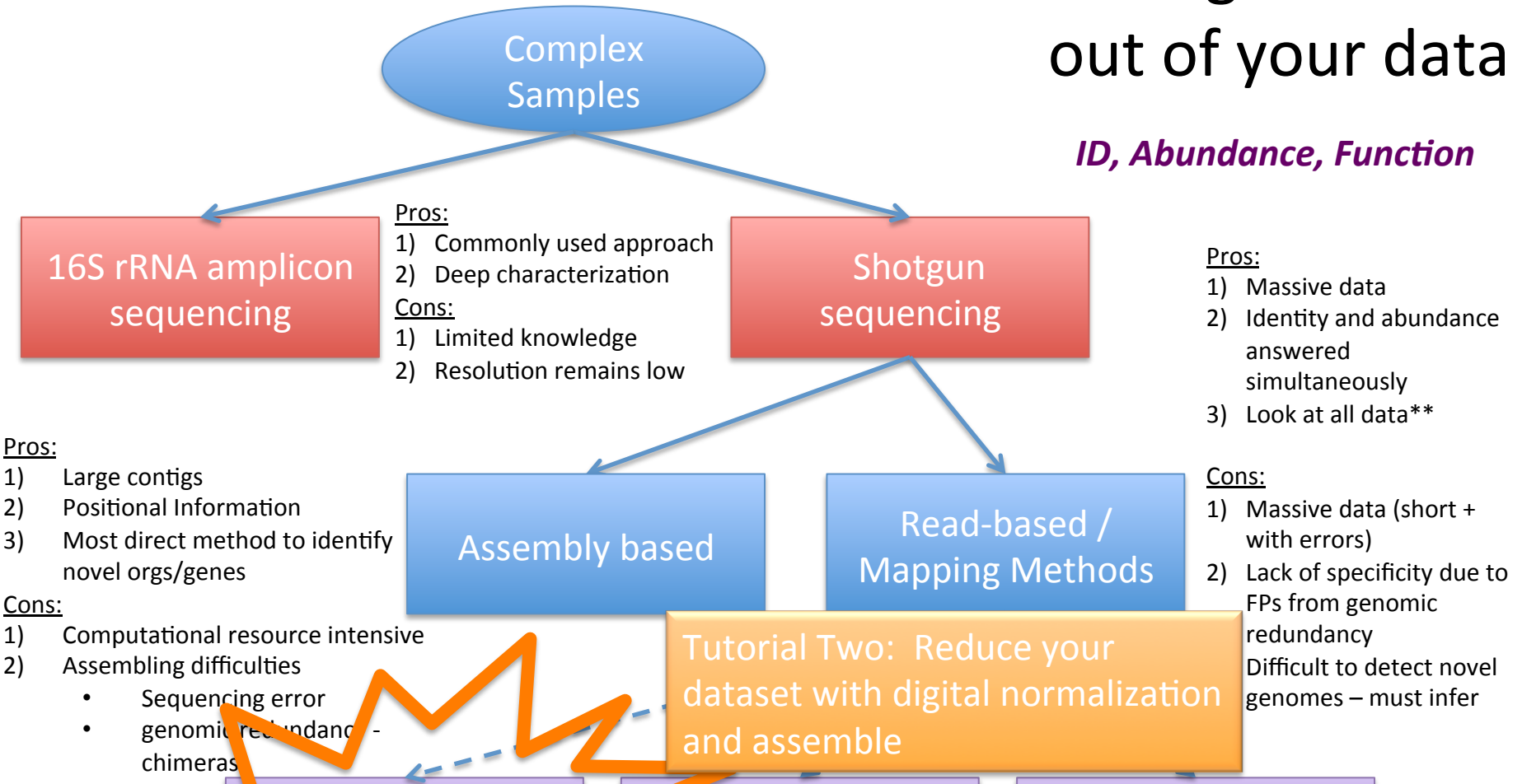

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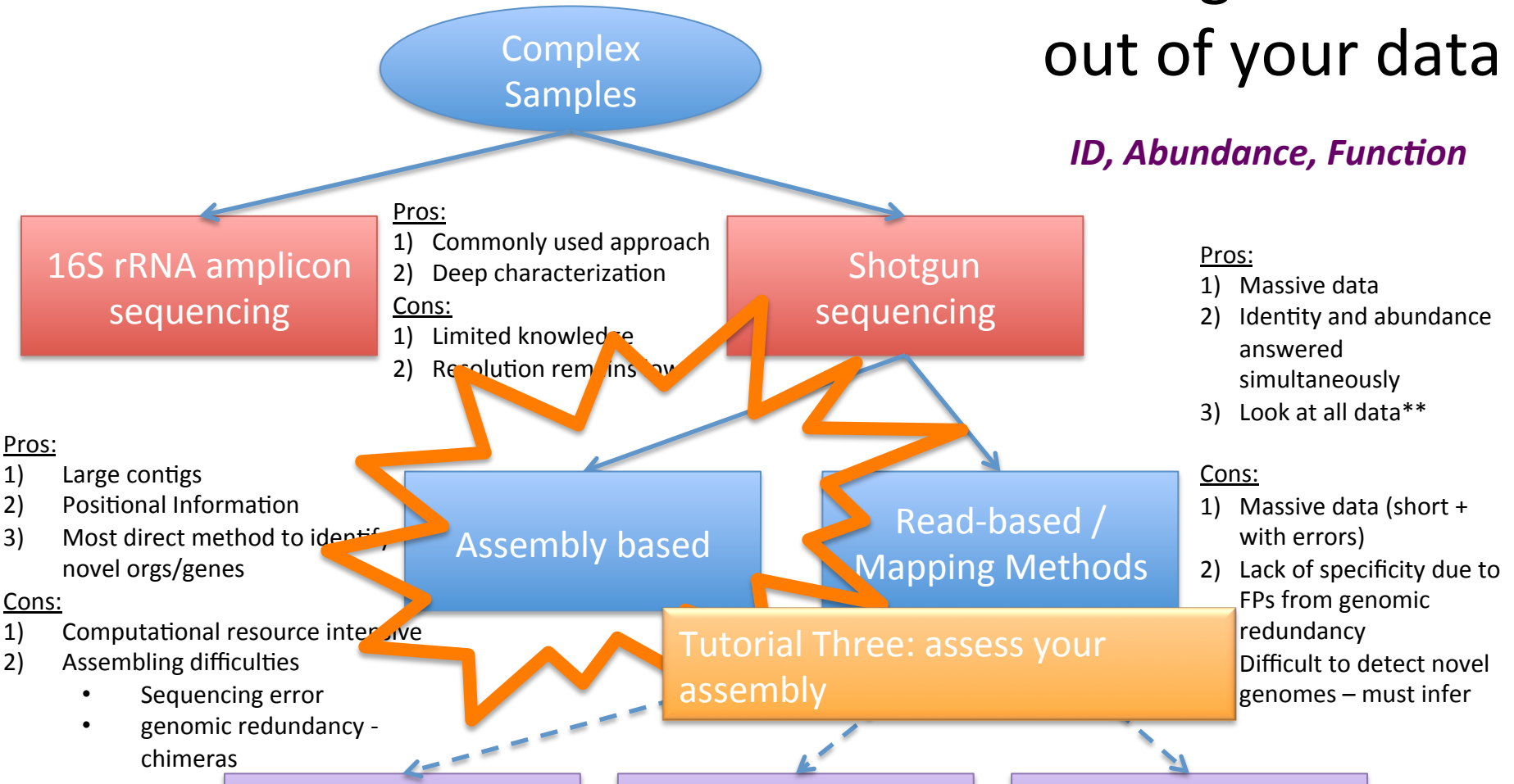

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Read-based / Mapping Methods

Tutorial Four: Mapping reads to estimate abundances

Query size reduction

a. Assembly / clustering
b. Unique K-mer

Reference database size reduction

a. Selection of marker genes
b. Identification of signatures (Kmers)

Faster search algorithms

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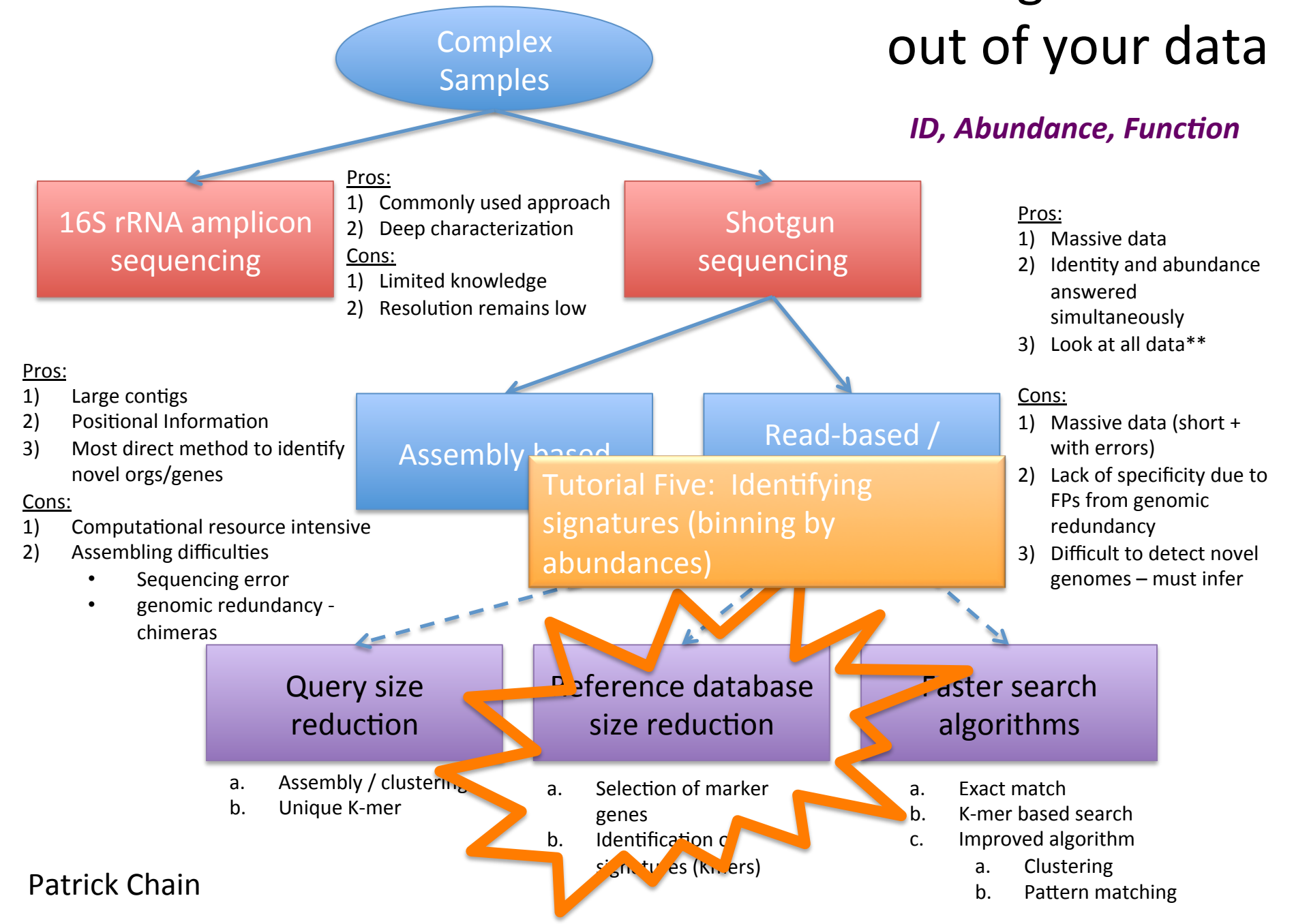

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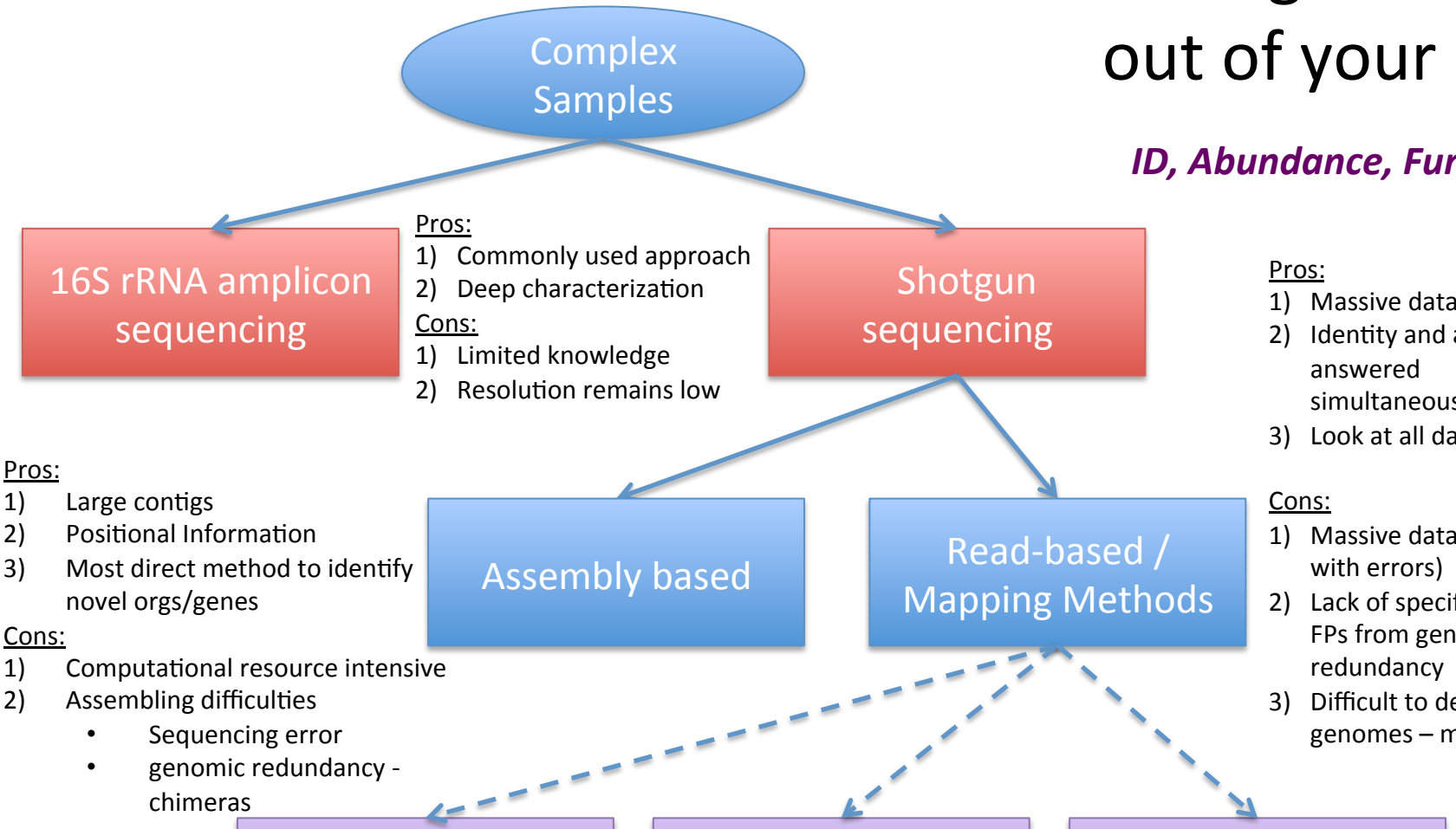

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So let’s do some learning…